

## Intraspecific Restriction Site Variation in the Chloroplast DNA of *Desmodium podocarpum* DC. (Leguminosae–Papilionoideae)

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We examined four restriction site changes of chloroplast DNA in 22 samples of five infraspecific taxa of *Desmodium podocarpum* DC. collected in China and Japan. Three patterns of distribution of restriction site changes are recognized, as were recognized in our previous study (Kajita and Ohashi 1994). All the obtained data are combined with those from 16 samples used in the previous study for further discussion. A newly added taxon from China, subsp. *szechuenense*, is included in the first clade together with samples of subsp. *podocarpum*, subsp. *fallax*, and Japanese samples of subsp. *oxyphyllum* var. *oxyphyllum*. As a result, samples from all the four subspecies are included in this clade. All the materials of var. *mandshuricum* are included in the second clade with three samples of var. *oxyphyllum*. Four newly added Chinese samples of var. *oxyphyllum* are included in the third clade with those from Kyushu and Taiwan. No materials from northern part of Japan are included in this clade, which suggests a possible geographical pattern in the distribution of this genome type. Materials of subsp. *szechuenense* and subsp. *oxyphyllum* from China are separated in different clades.

**Key words:** cpDNA, *Desmodium*, *Desmodium podocarpum*, phylogeny, subgenus *Podocarpium*

### Introduction

*Desmodium podocarpum* DC. (Leguminosae–Papilionoideae: tribe Desmodieae) and its allies form an extremely variable species group distributed in warm-temperate and temperate regions in eastern Asia. A number of different taxonomic treatments have been done for this species group by various authors (Nakai 1930, Isely 1951, Ohwi 1953, 1965a, 1965b, Ohashi 1973, Choi 1991, Yang and Huang 1995). In recent years, this species has often been treated under genus *Podocarpium* (Yang and Huang 1979, 1995, Cui et al. 1987, Huang 1998) or *Hylodes-*

*mum* (Ohashi 1999, Ohashi and Mill 2000), but we adopt *Desmodium* in this paper in convenience of continuation with our previous paper (Kajita and Ohashi 1994). Ohashi (1973) classified the species complex into one species with infraspecific taxa, based on the observation of many living materials as well as a large number of herbarium specimens. In addition to typical subspecies *podocarpum*, he recognized subsp. *oxyphyllum* (DC.) H.Ohashi and subsp. *fallax* (Schindl.) H.Ohashi. These three subspecies are mostly clearly distinguished by the shape of terminal leaflet. The three varieties ac-

commodated in subsp. *oxyphyllum* are var. *oxyphyllum*, var. *mandshuricum* Maxim., and var. *szechuenense* Craib. The former two are distinguished by the position of leaves on stem, habitat, and thickness of leaves, and are thought to be different only by populations or by individuals (Ohashi 1973). Var.

*szechuenense* can be clearly distinguished from the other varieties, and was recently treated as subsp. *szechuenense* (Craib) H.Ohashi, based on morphological characters, habitat, and distribution (Ohashi 1995). The infraspecific classification of *D. podocarpum* used in this study is shown in Table

Table 1. Infraspecific classification of *Desmodium podocarpum* DC., materials used in this study, and patterns of restriction site variations

sample	DNA	Score	Locality
<i>Desmodium podocarpum</i> DC. subsp. <i>podocarpum</i>			
CHINA	102	0110	Siding, Yongfu Co., Guangxi, CHINA (T. Nemoto et al. 1002029)
<i>Desmodium podocarpum</i> DC. subsp. <i>fallax</i> (Schindl.) H.Ohashi			
CHINA	104	0110	Hekou, Ziyuan Co., Guangxi, CHINA (T. Nemoto et al. 1006031)
<i>Desmodium podocarpum</i> DC. subsp. <i>szechuenense</i> (Craib) H.Ohashi			
CHINA	103	0110	Hekou, Ziyuan Co., Guangxi, CHINA (T. Nemoto et al. 1006036)
<i>Desmodium podocarpum</i> DC. subsp. <i>oxyphyllum</i> (DC.) H.Ohashi var. <i>oxyphyllum</i>			
MIYAGI1	263	0110	Sakunami, Sendai, Miyagi (T.Kajita & Y.Tateishi 92092701)
MIYAGI2	282	0110	Akiu, Sendai, Miyagi (T.Kajita & H.Ohashi 92101807)
MIYAGI3	284	0110	Aoshita, Sendai, Miyagi (T.Kajita & H.Ohashi 92101809)
MIYAGI4	285	0110	Aoshita, Sendai, Miyagi (T.Kajita & H.Ohashi 92101810)
MIYAGI5	286	0110	Jouge, Sendai, Miyagi (T.Kajita & H.Ohashi 92101811)
MIYAGI6	281	0001	Akiu, Sendai, Miyagi (T.Kajita & H.Ohashi 92101806)
MIE	261	0110	Shorenji, Nabari, Mie (T.Kajita 92092101)
NARA	260	0110	Seijoubou-keikoku, Nabari, Mie (T.Kajita 92092102)
NAGASAKI1	256	0110	Matsuyama-cho, Nagasaki (K.Yonekura 92848)
CHINA1	107	1100	Xinpu, Jianghua Co., Hunan, CHINA (H.Ohashi et al. 925120)
CHINA2	108	1100	Jinxiu, Jinxiu Co., Guangxi, CHINA (H.Ohashi et al. 929122)
CHINA3	109	1100	Zhangjiang, Rongshui Co., Guangxi, CHINA (T. Nemoto et al. 1003028)
CHINA4	110	1100	Hekou, Ziyuan Co., Guangxi, CHINA (H.Ohashi et al. 929122)
<i>Desmodium podocarpum</i> DC. subsp. <i>oxyphyllum</i> (DC.) H.Ohashi var. <i>mandshuricum</i> Maxim.			
MIYAGI1	276	0001	Akiu, Sendai, Miyagi (T.Kajita & H.Ohashi 92101801)
MIYAGI2	277	0001	Akiu, Sendai, Miyagi (T.Kajita & H.Ohashi 92101802)
MIYAGI3	278	0001	Akiu, Sendai, Miyagi (T.Kajita & H.Ohashi 92101803)
MIYAGI4	247	0001	Jouge, Sendai, Miyagi (T.Kajita 92071101)
MIE	246	0001	Kaochi-dani, Nabari, Mie (T.Kajita & H.Iketani 92091901)
NAGASAKI	253	0001	Tari-yama, Nagasaki (K.Yonekura 92846)

sample: abbreviation of sample. Samples in the text and Fig. 1 are shown by combination of last epithet of taxa and this abbreviation.

DNA: accession number of DNA sample.

Score: pattern of the four restriction site changes. Presence of restriction site is scored as 1, and absence as 0, in the order of restriction site change No. 3, 7, 9, 10. See text for more detail.

1.

In our previous work (Kajita and Ohashi 1994) we performed phylogenetic analysis using restriction site variation of chloroplast DNA for most species of *Desmodium* subgen. *Podocarpium*, and analyzed 16 samples from four infraspecific taxa of *D. podocarpum* except for subsp. *szechuenense*. Restriction site analysis of chloroplast DNA provided five restriction site changes which determined three groups that were not consistent with any infraspecific classification systems. The first group consisted of three subspecies: subsp. *podocarpum*, subsp. *fallax*, and subsp. *oxyphyllum*, while the second and third group contained only subsp. *oxyphyllum*. In subsp. *oxyphyllum*, however, only the second group included both var. *oxyphyllum* and var. *mandshuricum*, but the first and third only var. *oxyphyllum*. Considering the geographical distribution of samples of var. *oxyphyllum*, members of the first group were mostly distributed in northern Japan and the third in southern Japan. Var. *mandshuricum* was in a clade with some of var. *oxyphyllum* from southern Japan and Korea. As no samples from continental Asia were used, we have no information about the distribution pattern of each genome types in its wide distribution area. In addition, as samples used in the study were collected individually from distant populations, we have no information about the genomic composition in a rather small area where var. *mandshuricum* and var. *oxyphyllum* are in nearby populations.

In this paper, we report a result of restriction site analysis using four of the five restriction sites for 22 samples additional to the previous study. In the course of field researches in southern China in 1992 and 1993, we collected subsp. *szechuenense* that is endemic to China and the only taxon that was not included in the previous study. The questions we tried to solve are (1) in which group newly added Chinese samples will be

included; (2) do the groups recognized by the chloroplast genome have their own unique distribution pattern; and (3) whether the difference between var. *oxyphyllum* and var. *mandshuricum* are clear in a rather small area.

### Materials and Methods

Twenty-two samples of *Desmodium podocarpum* were collected in field researches in Japan and China in 1992 and 1993 (Table 1). Leaves were kept on ice until DNA extraction in laboratory. Seven samples were collected in Guanxi and Hunan Province of southern China. Ten materials were collected in Sendai city, Miyagi Prefecture. Five of them were collected from nearby populations in Akiu. We collected typical var. *oxyphyllum* and var. *mandshuricum* in this area. Voucher specimens are preserved in the herbarium of Tohoku University (TUS).

Total DNA was extracted from fresh leaves by CTAB method of Doyle and Doyle (1987). Southern blotting analysis of restriction site variations was performed as was described in Kajita and Ohashi (1994). We employed four of five restriction site changes (Nos. 3, 7, 9, and 10 in Table 2 and Fig. 1 of Kajita and Ohashi 1994) that were used to recognize three phylogenetic groups. The restriction site change No. 8 was not surveyed in this study because of a shortage of amount of several DNA samples. All restriction site changes were newly derived characters in the clade of *D. podocarpum* (Kajita and Ohashi 1994).

### Results and Discussions

We call the five infraspecific taxa of *Desmodium podocarpum* examined in this study arbitrarily by their last epithets in the following.

Restriction site changes examined in this study are shown in Table 1. The presence of site is scored as "1" and absence as "0". All the materials have one of the three patterns

of combinations of restriction site changes, that are 0110, 1100, and 0001, as were the same pattern recognized in the previous study (Kajita and Ohashi 1994). To compare these results with the previous data, distribution of the restriction site changes and the locality of all the samples listed in Table 1 are shown in a form of dendrogram (Fig. 1), together with data of 16 samples obtained in Kajita and Ohashi (1994).

A newly added taxon from China, *szechuenense*, is included in the clade which has restriction site change No. 9 together with samples of *podocarpum*, *fallax*, and Japanese samples of *oxyphyllum* (Fig. 1). As a result, samples from all the four subspecies are included in this clade. *Szechuenense* have been expected to be more closely related to *oxyphyllum* than *fallax* and *podocarpum*, and was treated as a variety of *D. podocarpum* subsp. *oxyphyllum* (Ohashi 1973). According to our observation in the fields and herbaria, plants of *szechuenense* are collected at river-side and have quite narrow leaflets and a robust root. These characteristics are often seen in a rheophyte. Considering these characteristics with the morphological resemblance between *szechuenense* and *oxyphyllum*, one possibility can be suggested that the former was locally (i. e., in China) derived from the latter. However, in this study, no Chinese samples of *oxyphyllum* are in the same clade with *szechuenense*, and *szechuenense* is more closely related to *podocarpum*, *fallax*, and Japanese samples of *oxyphyllum* (Fig. 1). The reason why such diversity of taxa and distribution are seen in a single clade is still in question. Phylogenetic relationships of *szechuenense* and other members that share restriction site change No. 9 should be studied with more samples using molecular marker with higher resolution.

Four newly added Chinese samples of *oxyphyllum* are included in the same clade with those from Kyushu and Taiwan (Fig. 1).

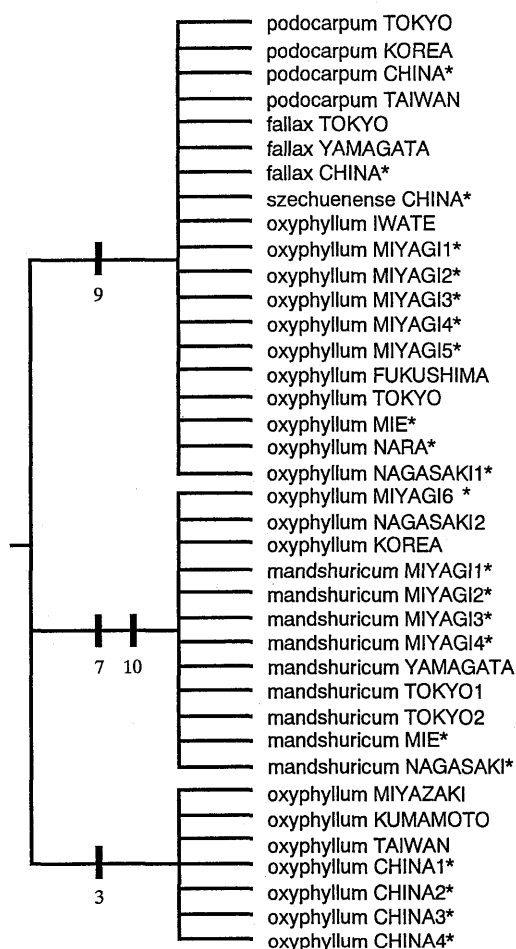


Fig. 1. Three groups of *Desmodium podocarpum* surveyed in this study and Kajita and Ohashi (1994). Vertical bars and numbers below show the shared restriction site changes that are synapomorphic characters of each clade and numbers designated to them in the previous work (Kajita and Ohashi 1994). Samples are shown by the combination of last epithet and the abbreviation of samples. Samples with asterisks are new in this study.

No materials from northern part of Japan are included in this clade. This result suggests a possible tendency of the geographical distribution of this chloroplast genome of *oxyphyllum* in South China, Taiwan, and Kyushu.

All the materials of *mandshuricum* which

are collected in a rather wide range of distribution area in Japan (from Miyagi, Mie and Nagasaki Prefectures) are included in the second clade (Fig. 1). Three samples (oxyphyllum MIYAGI6, oxyphyllum NAGASAKI2, and oxyphyllum KOREA) of *oxyphyllum* are also included in this clade. Although *mandshuricum* and *oxyphyllum* have been thought to be different only by populations or by individuals (Ohashi 1973), our results suggest a possibility that *mandshuricum*, which is generally represented in gross morphology, can be phylogenetically monophyletic, although presence of three samples of *oxyphyllum* included in this clade give a question about relationships between these varieties or about correct recognition of their morphological circumscription.

Oxyphyllum MIYAGI6 (Fig. 2b) was collected in a population found at Akiu in Sendai city. The leaves of the individual spread throughout the stem as in ordinary *oxyphyllum*, but they are thin in texture and pale whitish in lower surface as in

*mandshuricum*. This individual was grown in a place close to the population of typical *oxyphyllum* represented here by oxyphyllum MIYAGI2 (Fig. 2a) and of *mandshuricum* represented by mandshuricum MIYAGI1 (Fig. 2c). All were within 100 m in distance. In our molecular result, oxyphyllum MIYAGI2 is included in the first clade, but oxyphyllum MIYAGI6 is included in the second clade together with mandshuricum MIYAGI1. The presence of oxyphyllum MIYAGI6 in the second clade suggests several possibilities that the intermediate form like this material is a result of genetic exchange between *oxyphyllum* and *mandshuricum*, or that the morphological circumscription of *mandshuricum* should be extended. More detailed studies on other morphological differences of these groups as well as biosystematic studies will be necessary.

Our results show that samples from Kyushu, i. e., *mandshuricum* (NAGASAKI) and *oxyphyllum* (NAGASAKI1, NAGASAKI2, MIYAZAKI, KUMAMOTO) distribute throughout all the three clades

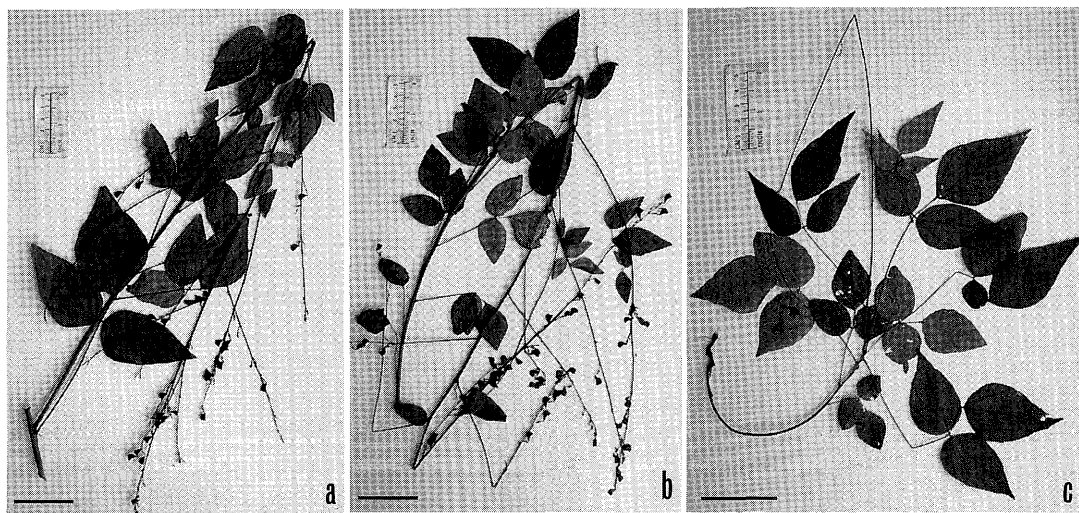


Fig. 2. Three individuals of *Desmodium podocarpum* subsp. *oxyphyllum* collected in nearby populations, in Sendai, Japan. a: var. *oxyphyllum* (oxyphyllum MIYAGI2), b: var. *oxyphyllum* (oxyphyllum MIYAGI6), c: var. *mandshuricum* (mandshuricum MIYAGI1). Voucher specimens in TUS. Scale bar = 5cm.

(Fig. 1). It seems likely that geographic differentiation is occurred between northern and southern part of Kyushu (Fig. 1). Analysis including more materials from Kyushu are necessary.

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梶田 忠<sup>a</sup>, 大橋広好<sup>b</sup>: マメ科ヌスビトハギ (広義) の葉緑体 DNA に見られる種内制限サイト変異

日本で普通に見られるはヌスビトハギ (広義) は、マメ科ヌスビトハギ亜属の中では最も北まで分布を広げた種であり、マルバヌスビトハギ (*D. podocarpum* subsp. *podocarpum*)、ケヤブハギ (subsp. *fallax*)、ヌスビトハギ (subsp. *oxyphyllum* var. *oxyphyllum*)、ヤブハギ (subsp. *oxyphyllum* var. *mandshuricum*)、そして中国に固有の四川山馬蝗 (中国名) subsp. *szechuenense* (以下 *szechuenense* と呼ぶ) という4亜種が知られている。著者ら (Kajita and Ohashi 1994) はヌスビトハギ亜属の系統関係を葉緑体 DNA の制限サイト変異を用いて研究した際、

日本、韓国、台湾から得られた *szechuenense* 以外のサンプルを用いて、ヌスビトハギ (広義) には3タイプの葉緑体ゲノムが存在し、それらの分布は現在の分類とは一致しないことを明らかにした。本研究では1992年と1993年に南中国マメ科調査などで得られた22サンプルを加え、葉緑体 DNA の種内変異をヌスビトハギ (広義) の全ての種内分類群と比較した。DNA の解析は Kajita and Ohashi (1994) で用いた方法に従った。また、そこで得られた4つの制限サイト変異のみを本研究に用いた。その結果、ヌスビトハギ (広義) の種内分類群は前報告の結果と同様、3グループに分かれる

ことが示された (Fig. 1). 最初のグループには, 1. 岩手県から長崎県までのヌスビトハギ, 2. 東京都, 山形県および中国のケヤブハギ, 3. 日本, 韓国, 台湾および中国のマルバヌスビトハギ, および 4. 中国の *szechuenense* が含まれた. 2 番目のグループには, 山形県から長崎県までのヤブハギと対馬, 鬱陵島 (韓国) および仙台市秋保のヌスビトハギとが含まれた. 3 番目のグループには, 宮崎県, 熊本県, 台湾および中国 (湖南省, 広西省) から得られた全てのヌスビトハギが含まれた. 中国固有の *szechuenense* は形態的にはヌスビトハギに近いが, 小葉の幅が著しく狭く, 地下

形が太く長く発達し, 川沿いに生育することから, ヌスビトハギから分化した溪流沿い植物である可能性が考えられる. しかし, *szechuenense* の葉緑体ゲノムは, ほぼ同所で採集したヌスビトハギを含む中国のヌスビトハギとは異なるグループに属し, この仮説は支持されなかった.

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